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Nuclear DNA amounts in European *Callitriche* species (Callitrichaceae)

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SUMMARY

Nuclear DNA amounts were determined for nine species, one subspecies and one hybrid of the European Callitrichaceae. The 2C DNA values ranged from 1.82 pg to 8.30 pg due to variation in chromosome numbers ($2n = 6-38$) and loss (maximally 40.9%) or amplification (maximally 28.5%) of the DNA amount. The results are discussed in relation to phylogeny.

Key-words: *Callitriche*, chromosomes, DNA amount, phylogeny.

INTRODUCTION

In Europe the genus *Callitriche* (Callitrichaceae) presently comprises at least 14 species of which 13 species and 1 subspecies are well known (Schotsman 1954, 1967, 1969, 1972a,b, 1982a; Dersch 1974; Schotsman & Andreas 1980; Schotsman & Haldimann 1981; Haldimann 1982; Cook 1983). The delimitation of the taxa is based on a combination of morphological and anatomical characters, floral biology, cytological data, ecology and geographical distribution. Two species and one subspecies live wholly submerged in water (called 'submerged'), two species live in water as submerged form or as form with floating or aerial rosettes ('aquatic') and nine species grow in water as submerged form or as form with floating rosettes and in humid soil as terrestrial form (called 'amphibious'). The intrageneric chromosome numbers range from $2n=6$ to $2n=38$. The most frequent number is $2n=10$ and the basic number (x) is 5 (see Table 1).

Species with $2n=10$ have characters in common with landplants (Schotsman 1982a) and most of these taxa can live wholly emerged. Because the species of this genus probably originated from terrestrial phanerogames (Arber 1920; Takhtajan 1959), $2n=2x=10$ could be the most primitive chromosome number of the genus.

There is some evidence that the submerged species are descendants of terrestrial taxa. The former species show, for instance, an important reduction in their morphological and anatomical characters (Schotsman 1982a). The species with $2n=8$ are either amphibious, aquatic or submerged, and species with $2n=6$ are submerged only (see Table 1). Therefore a reduction in characters seems to be coupled with a decrease in chromosome numbers. It is not known how the karyotype evolution towards $2n=6$ took place. All the chromosomes of the species with $2n=10$, 8 or 6 have distinct arms and intraspecific chromosomal polymorphism has been observed in some populations of *C. obtusangula* and *C. stagnalis* (Schotsman 1967).

The species with $2n=20$ have several characters in common with the $2n=10$ species. *C. platycarpa* ($2n=20$) arose by allopolyploidization according to Savidge (1960) but by

autopolyploidization according to Schotsman (1967). Which of these processes gave rise to the polyploidy of *C. palustris* ($2n=20$) is also unknown. *C. hybrid* ($2n=15$) is the only hybrid *sensu stricto* in this genus and probably originated from a cross between *C. cophocarpa* ($2n=10$) and *C. platycarpa* ($2n=20$; Savidge, 1959; Schotsman 1961b, 1967; Dersch 1974; Schotsman & Haldimann 1981). At least one of the species with 20 chromosomes is closely related to a species with 10 chromosomes.

C. brutia ($2n=28$) and *C. hamulata* ($2n=38$) are peculiar since they are morphologically almost similar (Schotsman 1967, 1982a). They could be delimited through their ecology, geography and chromosome numbers. They are allopolyploids according to Hedberg & Hedberg (1977).

Further evidence for systematic relationships within the genus *Callitriche* can be obtained by hybridization studies. Gene exchange between taxa, however, is generally prevented by various isolating mechanisms, one of the most important being the strong tendency to geitonogamy (Schotsman 1967, 1982a, b). The karyotypes have not, as yet, been analysed with the aid of chromosome-banding methods, cytophotometry or molecular techniques. Nuclear DNA amounts can be used to interpret the evolution of species. In this study the DNA contents of nine species, one subspecies and one hybrid are determined and the data contribute to the phylogenetic analysis of the European *Callitrichaceae*.

MATERIALS AND METHODS

Species and Provenance. The following *Callitriche* species were investigated.

C. truncata Guss. subsp. *occidentalis* (Rouy) Schotsm. Spain, Andalucia, Cota Doñana, 1984, H.D. Schotsman.

C. lusitanica Schotsm. Spain, Andalucia, Las Habas, 1984, H.D. Schotsman.

C. regis-jubae Schotsm. Spain, Province of Cádiz, Castellar de la Frontera, 1981, H.D. Schotsman.

C. obtusangula Le Gall. (a) France, Department of Cher, Clémont, 1981, H.D. Schotsman. Population with standard karyotype (without polymorphism). (b) Idem, 1981, in culture by H.D. Schotsman.

C. stagnalis Scop. France, Department of Cher, Cerdon, 1981, H.D. Schotsman. Population with standard karyotype (without polymorphism).

C. cophocarpa Sendtn. Switzerland, Jura, Les Seignolis, 1982, Haldimann.

C. hybrid. France, Jura, La Verrerie, 1982, G. Haldimann.

C. platycarpa Kütz. (a) Switzerland, Jura, La Ronde, 1982, G. Haldimann. (b) France, Department of Cher, La Chapelotte, 1981, H.D. Schotsman.

C. palustris L. Spain, Central Pyrenees, Province of Huéscas, Valley of Esera, 1984, G. Haldimann.

C. brutia Pet. Ireland, West Cork, Kilcrohane, 1981, B. van Zanten (identified by H.D. Schotsman).

C. hamulata Kütz., ex Koch. France, Department of Cher, Nançay, 1981, H.D. Schotsman.

Some species are rare or occupy a limited area. Specimens of *C. hermaphroditica* L., *C. pulchra* Schotsm., *C. cribrosa* Schotsm. and *C. lenisulca* Clav. could not be discovered during recent years.

Methods. The specimens, collected in nature (*C. obtusangula* also cultured), were fixed in 3:1 absolute ethanol:glacial acetic acid (v/v) and stored in a deepfreeze until further

processing. Within three months after fixation the plants were hydrolysed in 5N HCl at room temperature for 25 min and stained by the Feulgen-reaction for 2 h. Root tips and/or parts of young leaves were squashed in 45% acetic acid and the slides made permanent in euparal by the freeze method.

The nuclear DNA content in arbitrary units was measured using a Zeiss MPM 01 microscope photometer with equipment as described by Tempelaar (1980). The nuclei of at least three plants from each sample of one species were measured. Since the specimens were collected over 4 years, plants from one species of which the DNA amount had already been established, were collected again in the next year of sampling. Measurements on the nuclei of these plants and of a potato (see below) were used as a control to obtain standardized results. The nuclei of tissues displaying mitoses were measured only. The 2C DNA value was calculated from the measurements of interphase nuclei in G_1 . That the values were obtained from G_1 nuclei, was established by comparing them with the values of prophase and telophase which were considered to be 4C and $2 \times 2C$, respectively.

The relative DNA values were corrected to picograms using the absolute DNA amount of interphase nuclei of root tips of the interdihaploid potato clone H²578 ($2n=2x=24$; 2C DNA = 1.80 pg) as a standard (Jacobsen *et al.* 1983; cf. Bennet & Smith 1976). The root tips were fixed and processed in the same way as the test material. The interphase structures of this potato are rather similar to those of the species of *Callitriche* and thus suitable as reference.

RESULTS AND DISCUSSION

The results of the DNA measurements of interphase nuclei are summarized in Table 1. The amounts of DNA in absolute units are presented at the 2C level and per chromosome. A 2C amount of about 2.8 pg DNA was found in three out of four amphibious species with 10 chromosomes, namely *C. regis-jubae*, *C. stagnalis* and *C. cophocarpa*. If, as mentioned in the Introduction, 10 chromosomes are the ancestral diploid chromosome number (basic number $x=5$), then it may be assumed that 2.84 pg DNA (mean amount) is the original 2C amount of the species of *Callitriche*. Starting from the correctness of this assumption, the observed values represent valuable information (for reviews see Price 1976; Bennett & Smith 1976; Flavell 1982; Walbot & Cullis 1985).

The original amount is still present in *C. cophocarpa*, from which the values of *C. stagnalis* and *C. regis-jubae* do not differ significantly (*t*-tests: $P>0.05$). These species differentiated (cf. Schotsman 1967, 1977) without changing their nuclear DNA amounts. *C. obtusangula* is the only species with 10 chromosomes with a significantly different, namely a 28.5% higher, amount of DNA (*t*-test: $P<0.01$). Increase or decrease in nuclear DNA content has been recorded before for related plant species with similar chromosome numbers and has been explained as being due to amplification or deletion of base sequences, respectively, by unknown programs (Price 1976; Flavell 1982; Walbot & Cullis 1985). It is not known where the amplification took place in the chromosomes of *C. obtusangula*. This species differs from *C. regis-jubae*, *C. stagnalis* and *C. cophocarpa* in pollen shape and in a rare anatomical character of the pericarp (Schotsman & Andreas 1974; Schotsman 1977). Moreover, the pollen grains of *C. obtusangula* are not recognized by the stigmata of *C. stagnalis* and vice versa (inhibition of pollen germination and pollen tube growth), though these plants have a similar pollination (Schotsman, unpublished). These facts provide evidence for a remarkable phylogenetic distance which is sustained by and may have occurred through the increase in nuclear DNA.

Table 1. DNA amounts of European species of *Callitriche*

Species	Form	Number of nuclei	2C nuclear DNA amount/pg (\pm SD)	Chromosome number (2n)	DNA amount per chromosome = chromatid/pg
<i>C. truncata</i> subsp.					
<i>occidentalis</i>	submerged	50	2.61 \pm 0.13	6	0.44
<i>lusitanica</i>	aquatic	82	1.82 \pm 0.11	8	0.23
<i>regis-jubae</i>	amphibious	59	2.88 \pm 0.15	10	0.29
<i>C. obtusangula</i> *					
(Cher)	amphibious	88	3.65 \pm 0.21	10	0.36
(culture)	amphibious	75	3.66 \pm 0.19	10	0.37
<i>C. stagnalis</i> *	amphibious	63	2.80 \pm 0.14	10	0.28
<i>C. cophocarpa</i> *	amphibious	89	2.84 \pm 0.18	10	0.28
<i>C. hybrid</i>	amphibious	68	4.14 \pm 0.19	15	0.28
<i>C. platycarpa</i> *					
(Jura)	amphibious	51	5.52 \pm 0.21	20	0.28
(Cher)	amphibious	58	5.55 \pm 0.18	20	0.28
<i>C. palustris</i> *	amphibious	94	3.38 \pm 0.16	20	0.17
<i>C. brutia</i>	amphibious	74	5.46 \pm 0.20	28	0.20
<i>C. hamulata</i> *	amphibious	125	8.30 \pm 0.30	38	0.22

Not available: *C. hermaphroditica**, submerged, 2n=6; *C. pulchra*, submerged, 2n=8; *C. cribrosa*, amphibious, 2n=8; *C. lenisulca*, aquatic, 2n=10.

*Dutch species; *C. cophocarpa* very rare, last record from 1930.

The DNA value of *C. lusitanica* (2n=8) indicates a loss of 36.2% of DNA and the value of *C. truncata* subsp. *occidentalis* (2n=6) a loss of 8.2% DNA compared with the original 2C amount. (The 2C value of *C. truncata* differs significantly from that of *C. stagnalis*; *t*-test: $P < 0.01$). The evolution towards lower chromosome numbers is thus not followed by a proportional diminution in nuclear DNA content. A decrease in the basic number of chromosomes may occur by either centric (= Robertsonian) fusion of acro- or telocentric chromosomes (John & Lewis 1968) or meiotic missegregation of multivalents in double interchange heterozygotes (Schubert & Rieger 1985). In both situations the genome loses one translocated chromosome which is formed by non-essential (short) arms. Though not evidenced by the shape of the chromosomes (acrocentrics only; Schotsman 1967), such a loss must have occurred once in the genome of *C. lusitanica* and twice in the genome of *C. truncata* subsp. *occidentalis*. The slight reduction in the DNA content of *C. truncata* subsp. *occidentalis* could then have been the result of the loss of two non-essential translocated chromosomes per genome (cf. Marks 1983). However, *C. lusitanica* has lost much more DNA than can be explained by the loss of one translocated chromosome. This means that the genome lost a large amount of other dispensable DNA (cf. Price 1976; Flavell 1982; Walbot & Cullis 1985). The evolution from species with terrestrial characters with 2n=10 towards submerged species with lower chromosome numbers, as noted in the Introduction, is thus accompanied by a complex karyotype evolution.

C. platycarpa (2n=20) has double the original quantity of nuclear DNA, which is in accordance with the presence of polyploidy. This species is considered to be an allotetraploid of *C. cophocarpa* and *C. stagnalis* by Savidge (1960), but may equally be an autotetraploid of *C. cophocarpa* according to Schotsman (1967). The DNA amount (per chromosome) indicates that the ancestral parents were among the species with 10 chromosomes and about 2.8 pg DNA, to which *C. cophocarpa* as well as *C. stagnalis* belong. It

remains to be solved whether *C. platycarpa* has a hybrid origin or not. It is the only polyploid species which retained the original amount of DNA per chromosome.

The sterile natural hybrid *C. hybrid* ($2n=15$), collected in the Jura, probably originated from a cross between *C. cophocarpa* ($2n=10$) and *C. platycarpa* ($2n=20$), according to Schotsman (1967) and Schotsman & Haldimann (1981). The DNA measurements do not conflict with this assumption, for the DNA amount per chromosome (0.28 pg) of the hybrid is similar to that of the parental species.

Compared with *C. platycarpa*, *C. palustris* ($2n=20$) has a much lower amount of DNA. The content per chromosome (0.17 pg) is the lowest of all species measured. This species is probably tetraploid because the chromosomes are acrocentric and rather similar to those of the $2n=10$ species (Schotsman 1954, 1967). If so and if the ancestor(s) had 10 chromosomes with about 2.8 pg DNA, the genomes of this species lost 40.9% DNA without loss of chromosomes. *C. palustris* also differs in some traits, e.g. in the pollination processes, from the other species with $2n=10$ and 20 chromosomes (Schotsman 1954, 1982a) and thus is clearly separated phylogenetically.

Hedberg & Hedberg (1977) suppose that the chromosome numbers of *C. brutia* ($2n=28$) and *C. hamulata* ($2n=38$) arose through hybridization between an afromontane species with $2n=18$ and a species with $2n=10$ and $2n=20$, respectively, followed by doubling of the chromosomes, but Schotsman (1982b) doubts such an allopolyploid origin. The total quantities of DNA point to polyploidy. The DNA content per chromosome of these species is low and a loss of 36.0% and 27.0% DNA took place at the hexaploid level for *C. brutia* and at the octoploid level for *C. hamulata*, respectively. In both species it is likely that diminution of DNA is due to Robertsonian fusion or meiotic missegregation, as described above, because of the presence of a pair of metacentric chromosomes and hypoploidy for two chromosomes (Schotsman 1961a,b, 1967; Haldimann 1982). Because the metacentrics are found in both species and have a normal meiotic behaviour, the interchanges apparently took place in one of the ancestral parents and not since the origin of these species. Whatever may have happened, the quantity of DNA lost in this way equals the amount of two non-essential translocation chromosomes in both species. The high percentages of loss indicate that somewhere in the genomes DNA diminution not due to chromosomal rearrangements has taken place. Because *C. brutia* and *C. hamulata* have several characters in common, some authors (Clapham *et al.* 1962; Perring & Walters 1962) concluded that these callitriches are subspecies (of *C. intermedia* Hoffm.). However, Schotsman (1954, 1958, 1967) and Haldimann (1982) considered them as species, which is supported by the differences in loss of DNA content per genome.

The species *C. lusitanica*, *C. palustris* and *C. brutia* have to pass a short generation time for climatological and ecological reasons (Schotsman 1982a). They are equipped with a low DNA content per chromosome. The nuclear DNA content of a plant is correlated with the minimum generation time of that plant according to Bennett (1972). Consequently, these three taxa may have lost DNA as an adaptation to a rapid development (cf. Grime & Mowforth 1982). *C. antarctica* Engelm. ($2n=40$) from the sub-Antarctic island South Georgia has a lower DNA content (0.23 pg DNA per chromosome) in order to survive in a cold environment (Bennett *et al.* 1982). This may also apply to *C. hamulata* which prefers a cold water habitat (Schotsman 1967).

The present study demonstrates that the European taxa of *Callitriche* have a more complex evolutionary history than previously supposed. The evolutionary relationships between the species can be explored further if the karyotypes are analysed first with chromosome banding techniques.

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